110> APPLICANT: KUWABARA, HASHIGUCHI, Kenichi NAKAMATSU, Tsuyoshi KURAHASHI, Osamu MORI, Yukiko ITO, Hisao 120> TITLE OF INVENTION: CARBAMOYL-PHOSPHATE SYNTHETASE GENE OF CORYNEFORM BACTERIA AND METHOD FOR PRODUCING L-ARGININE 130> FILE REFERENCE: OP945CIP 140> CURRENT APPLICATION NUMBER: US/09/629,616 141> CURRENT FILING DATE: 2000-07-31 160> NUMBER OF SEQ ID NOS: 7 170> SOFTWARE: PatentIn Ver. 2.0 210> SEQ ID NO 1 211> LENGTH: 4837 212> TYPE: DNA 213> ORGANISM: Brevibacterium lactofermentum 220> FEATURE: 221> NAME/KEY: CDS 222> LOCATION: (283)..(1461) 221> NAME/KEY: CDS 222> LOCATION: (1470)..(4808) 400> SEQUENCE: 1 gatccaggaa aaacctggac agcatccggt gcagactttg cgtccaaggo tgaaaacacc 60 ccatttgagg gccaggaatt cagcgctaag gtcacacaca ccgtgcttcg tggcaaggtg 120 acttgtgcag acggagttgc gcaagacgct taacgggtgg gtgcatagta tgcacgcgcc 180 gcattgcata taatgcaatg aattgaataa actagattca gggttatcaa ccagccaatt 240 tcttttaaaa agacagacac acgaaaggcg acaacagtca cc gtg agt aaa gac Val Ser Lys Asp ace ace ace tac cag gga gte ace gag ate gga tee gtt eeg gea tac Thr Thr Thr Tyr Gln Gly Val Thr Glu Ile Gly Ser Val Pro Ala Tyr 15 10 ctg gtt ctt gca gac gga cgt agc ttc acc gga ttt ggc ttt gga gct 390 Leu Val Leu Ala Asp Gly Arg Thr Phe Thr Gly Phe Gly Phe Gly Ala 30 25 atc ggc acc acc ctt ggt gag gca gtg ttc acc acc gcc atg acc ggt Ile Gly Thr Thr Leu Gly Glu Ala Val Phe Thr Thr Ala Met Thr Gly 438 45 40 tac caa gaa acc atg acc gat cct tcc tat cac cgc cag att gtt gtg Tyr Gln Glu Thr Met Thr Asp Pro Ser Tyr His Arg Gln Ile Val Val 486 60 55 get acc gca cca cag aftc ggt aac acc ggc tgg aac gat gag gac aac 534 Ala Thr Ala Pro Gln #le Gly Asn Thr Gly Trp Asn Asp Glu Asp Asn 75 gag too ogo gao gg $ot\!\!\!/$ aag att tgg gtt goa ggo ott gtt ato ogo gao 582 Glu Ser Arg Asp Gy Lys Ile Trp Val Ala Gly Leu Val Ile Arg Asp 95 90 ctc gca gca cgt/gtg tcc aac tgg cgc gcc acc acc tcc ttg cag cag 630 Leu Ala Ala Arg Val Ser Asn Trp Arg Ala Thr Thr Ser Leu Gln Gln 115 110 gaa atg gca gac caa ggc atc gtc ggc atc ggc gga atc gac acc cgc 678 Glu Met Ala Asp Gln Gly Ile Val Gly Ile Gly Gly Ile Asp Thr Arg 130 125 120 gca ctg gt# cgc cac ctg cgc aac gaa ggt tcc atc gca gcg ggc atc 726 Ala Leu Van Arg His Leu Arg Asn Glu Gly Ser Ile Ala Ala Gly Ile 145 140 774 ttc tcc ggc gct gac gca cag cgc cca gtt gaa gaa ctc gta gag atc Phe Ser/Gly Ala Asp Ala Gln Arg Pro Val Glu Glu Leu Val Glu Ile 160 155 gtc aag aat cag cca gca atg acc ggc gca aac ctc tcc gtt gag gtc Val Lys Asn Gln Pro Ala Met Thr Gly Ala Asn Leu Ser Val Glu Val 175 170 tot got gat gaa acc tac gtc atc gaa gct gag ggc gaa gag cgc cac 870

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	tcc Ser	cgc Arg	gtc Val	ggc Gly 605	tac Tyr	gaa Glu	act Thr	gtc Val	atg Met 610	gtc	aac Asn	tg¢ Cys	aac Asn	cca Pro 615	gag	acc Thr	3320
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	acc Thr	ttc Phe 635	gaa	gac Asp	gtc Val	atg Met	gag Glu 640	gtc	tac Tyr	cac His	gct Ala	gag Gly 646	gcg	cag Gln	tcc Ser	ggc	3416
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	tta	gca Ala	gat Asp	cgt Arg	ttg Leu 670	aag	aag Lys	gct Ala	C1% ggc/	gte Val 675	cct Pro	gtc Val	att	ggt Gly	acc Thr 680	tcc Ser	3512
	cca Pro	gag Glu	gca Ala	atc Ile 685	Asp	atg Met	gct Ala	gag Glu	gac Asp 690	cgt Arg	ggc Gly	gag Glu	ttc Phe	ggt Gly 695	Ala	a ctg a Leu	3560
	ctg Leu	aac Asn	cgc Arg	gag Glu	cag	ctt Leu	cct Pro	ggt Ala 705	Pro	gca Ala	ttc Phe	ggc Gl	acc Thr	Ala	a aco	tct Ser	3608
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I	1e	Arg	Ala	Ala	Ala 1070	Val	Thr	Vál	Gly	Val 1075	Pro	Let	ı Ile	e Thr	Th:	r Val O	4760
G	ln	Gly	Val	Thr 1085	Ala	Ala	Va1	Glr	Gly 1090	r Ile	Glu	ı Ala	a Lev	1 Arg	g Glı	g ggc	
g V	tt al	Val	ago Ser 1100	Val	cgc Arg	gcg Ala	g ¢tg Leu	cag Glr 1105	ı Glu	t cto Let	gac Asp	c cac His	gca Ala 1110	a Va.	c aag l Ly	g gct s Ala	4808
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7	/al 1	. Ser	Lys	s Asp	/ !	5				10)				1	y Ser .5	
)ر2	Ó				2	5				3	0	y Phe	
			35	5 /				4	0				4	5		r Thr	
		50)	/			5!	5				6	0			s Arg	
· ·	G1r 65		e va.	ι va.	ı Al	7 T		a Pf	U G1	11 11	7		11 111	GI	y 11	p Asn 80	

Asp Glu Asp Asn Glu Ser Ang Asp Gly Lys Ile Trp Val Ala Gly Leu 85 90 · Val Ile Arg Asp Leu Ala Ala Arg Val Ser Asn Trp Arg Ala Thr Thr 100 105 Ser Leu Gln Gln Glu Met Ala Asp Gln Gly Ile Val Gly Ile Gly Gly 120 Ile Asp Thr Arg Ala Leu Val Arg His Leu Arg Asn Glu Gly Ser Il**g** 135 140 Ala Ala Gly Ile Phe Ser Gly Ala Asp Ala Gln Arg Pro Val Glu 💋 lu 150 155 Leu Val Glu Ile Val Lys Asn Gln Pro Ala Met Thr Gly Ala AsA Leu 170 Ser Val Glu Val Ser Ala Asp Glu Thr Tyr Val Ile Glu Ala Álu Gly 185 Glu Glu Arg His Thr Val Val Ala Tyr Asp Leu Gly Ile Lys Gln Asn 195 200 205 Thr Pro Arg Arg Phe Ser Ala Arg Gly Val Arg Thr Val /Ile Val Pro 215 220 Ala Glu Thr Pro Leu Glu Asp Ile Lys Gln Tyr Asn Pro Ser Gly Val 230 235 Phe Ile Ser Asn Gly Pro Gly Asp Pro Ala Ala Ala Asp Val Met Val 250 245 Asp Ile Val Arg Glu Val Leu Glu Ala Asp Ile / Pr/o Phe Ph/e Gly Ile 260 265 Cys Phe Gly Asn Gln Ile Leu Gly Arg Ala Phe Gly Met Glu Thr Tyr 285 280 Lys Leu Lys Phe Gly His Arg Gly Ile Asn Val Pro Val Lys Asn His 295 Asm His Gly Phe Ala Leu Ile Thr Gly Lys Ile Asp Ile Thr Ala Gla 315 310 Lys Gly Glu Ala Gly Gln Glu Phe Glu Thr Asp Phe Gly Thr Ala Ile 32/0 325 Val Thr His Thr Cys Leu Asn Asp Gly 1/2 Val Glu Gly Val Ala Leu 345 340 Lys Ser Gly Arg Ala Tyr Ser Val Gly Tyr His Pro Glu Ala Ala Ala 360 Gly Pro Asn Asp Ala Ser Pro Leu Phe Asp Gln Phe Val Glu Leu Met 375 Asp Ala Asp Ala Gln Lys Lys Gly Ala 390

<210> SEQ ID NO 3 <211> LENGTH: 1113

<212> TYPE: PRT

<213> ORGANISM: Brevibacterium /actofermentum

<400> SEQUENCE: 3

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His Glu Thr Val Ala Glu Leu Gly Leu Pro Val Val Val Arg Pro Ser - Phe Thr Met Gly Gly Leu Gly Ser Gly Leu Ala Tyr Asn Thr Glu Asp Leu Glu Arg Ile Ala Gly Gly Gly Leu Ala Ala Ser Pro Glu Ala Asn Val Leu Ile Glu Glu Ser Ile Leu Gly Trp Lys Glu Phe Glu Leu Glu Leu Met Arg Asp Thr Ala Asp Asn Val Val Ile Cys Ser Ile Gly Asn Val Asp Ala Leu Gly Val His Thr Gly Asp Ser Val Thr Val Ala Pro Ala Leu Thr Leu Thr Asp Arg Glu Phe Gln Lys Met Arg Asp Gln Ile Gln Phe Ala Ile Asn Pro Val Asp Gly Arg Ile Ile Thr Ile Glu Met Asn Pro Arg Val Ser Arg Ser Ser Ala Leu Ala Ser/Lys Ala Thr Gly Phe Pro Ile Ala Lys Met Ala Ala Lys Leu Ala Ile Gly Tyr Thr Leu Asp Glu Ile Thr Asn Asp Ile Thr Gly Glu Thy Pro Ala Ala Phe Glu Pro Thr Ile Asp Tyr Val Val Val Lys Ala/Pro Arg Phe Ala Phe Glu Lys Phe Val Gly Ala Asp Asp Thr Leu 7hr/Thr Thr Met Lys Ser Val Gly Glu Val Met Ser Leu Gly Arg Asn Tyr Ile Ala Ala Leu Asn 95 Lys Ala Leu Arg Ser Leu Glu Thr Lys Gln Gly Phe Trp Thr Lys 41Ø Pro Asp Glu Phe Phe Ala Gly Glu Arg A/a Thr Asp Lys Ala Ala Val Leu Glu Asp Leu Lys Arg Pro Thr Glu/Gly Arg Leu Tyr Asp Val Glu Leu Ala Met Arg Leu Gly Ala Ser Val Glu Glu Leu Tyr Glu Ala Ser Ser Ile Asp Pro Trp Phe Leu Ala Glu Leu Glu Ala Leu Val Gln Phe Arg Gln Lys Leu Val Asp Ala Pro Phe Leu Asn Glu Asp Leu Leu Arg Glu Ala Lys Phe Met Gly Leu Ser Asp Leu Gln Ile Ala Ala Leu Arg Pro Glu Phe Ala Gly Glu Asp Gly Val Arg Thr Leu Arg Leu Ser Leu Gly Ile Arg Pro Val Phe Lys Thr Val Asp Thr Cys Ala Ala Glu Phe Glu Ala Lys Thr Pro Tyr His Tyr Ser Ala Tyr Glu Leu Asp Pro Ala Ala Glu Ser Glu Val Asía Pro Gln Thr Glu Arg Glu Lys Val Leu Ile Leu Gly Ser Gly Pro/Asn Arg Ile Gly Gln Gly Ile Glu Phe Asp Tyr Ser Cys Val His Ala Leu Glu Leu Ser Arg Val Gly Tyr Glu Thr Val Met Val Asn $\not{
m c}$ ys Asn Pro Glu Thr Val Ser Thr Asp Tyr Asp Thr Ala Asp Arg Ley Tyr Phe Glu Pro Leu Thr Phe Glu Asp Val Met Glu Val Tyr His Ala Glu Ala Gln Ser Gly Thr Val Ala Gly Val Ile Val Gln Leu Gly/Gly Gln Thr Pro Leu Gly Leu Ala Asp Arg Leu Lys Lys Ala Gly Va/ Pro Val Ile Gly Thr Ser Pro Glu Ala Ile Asp Met Ala

Glu Asp Arg Gly Glu Phe Gry Ala Leu Leu Asn Arg Glu Gln Leu Pro •690 Ala Pro Ala Phe Gly Thr Ala Thr Ser Phe Glu Glu Ala Arg Thr Val 710 Ala Asp Glu Ile Ser Tyr Pro Val Leu Val Arg Pro Ser Tyr Val Leu 725 730 Gly Gly Arg Gly Met Glu Ile Val Tyr Asp Glu Ala Ser Leu Glu Asp 740 745 Tyr Ile Asn Arg Ala Thr Glu Leu Ser Ser Asp His Pro Val Leu **Y**al 760 765 Asp Arg Phe Leu Asp Asn Ala Ile Glu Ile Asp Val Asp Ala Ley Cys 770 775 780 Asp Gly Asp Glu Val Tyr Leu Ala Gly Val Met Glu His Ile ှ u Glu 790 795 Ala Gly Ile His Ser Gly Asp Ser Ala Cys Ala Leu Pro Pro/Met Thr 810 Leu Gly Ala Gln Asp Ile Glu Lys Val Arg Glu Ala Thr Lys Lys Leu 820 825 Ala Leu Gly Ile Gly Val Gln Gly Leu Met Asn Val Gln/Tyr Ala Leu 840 Lys Asp Asp Ile Leu Tyr Val Ile Glu Ala Asn Pro Ard Ala Ser Arg 855 860 Thr Val Pro Phe Val Ser Lys Ala Thr Gly Val Asn Ley Ala Lys Ala 875 870 Ala Ser Arg Ile Ala Val Gly Ala Thr Ile Lys Asp/Leu Gln Asp Glu 895 890 885 Gly Met Ile Pro Thr Glu Tyr Asp Gly Gly Ser Leu Pro Leu Asp Ala 900 905 Pro Ile Ala Val Lys Glu Ala Val Leu Pro Phe Asn Arg Phe Arg Arg 925 920 Pro Asp Gly Lys Thr Leu Asp Thr Leu Leu Ser Pro Glu Met Lys Ser 935 940 Thr Gly Glu Val Met Gly Leu Ala Asn Asn Phe Gly Ala Ala Tyr Ala 955 950 Lys Ala Glu Ala Gly Ala Phe Gly Ala Ley Pro Thr Glu Gly Thr Val 965 970 Phe Val Thr Val Ala Asn Arg Asp Lys Arg Thr Leu Ile Leu Pro Ile 985 980 Gln Arg Leu Ala Ser Met Gly Tyr Lys/Ile Leu Ala Thr Glu Gly Thr 1000 1005 Ala Gly Met Leu Arg Arg Asn Gly Ile Asp Cys Glu Val Val Leu Lys 1020 1015 Ala Ser Asp Ile Arg Glu Gly Val Glu Gly Lys Ser Ile Val Asp Arg 1035 1030 Ile Arg Glu Gly Glu Val Asp Ley Ile Leu Asn Thr Pro Ala Gly Ser 1050 1045 Ala Gly Ala Arg His Asp Gly Tyr Asp Ile Arg Ala Ala Ala Val Thr 1060 1065 Val Gly Val Pro Leu Ile Thr∥Thr Val Gln Gly Val Thr Ala Ala Val 1080 1085 1075 Gln Gly Ile Glu Ala Leu A#g Glu Gly Val Val Ser Val Arg Ala Leu 10/95 1100 Gln Glu Leu Asp His Ala Wal Lys Ala 1110

<210> SEQ ID NO 4

<211> LENGTH: 32

<212> TYPE: DNA

<213> ORGANISM: Artificia∜ Sequence

<220> FEATURE:

<223> OTHER INFORMATION:/Description of Artificial Sequence:primer for amplifying kanamydin resistant gene of Streptococcus faecalis

<400> SEQUENCE: 4

cccgttaact gcttgaaacc caggacaata ac

<210> SEQ ID NO 5 <211> LENGTH: 30 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence:primer for amplifying kanamycin resistant gene of Streptococcus faecalis <400> SEQUENCE: 5 cccgttaaca tgtacttcag aaaagattag 30 <210> SEQ ID NO 6 <211> LENGTH: 26 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence:primer amplifying Escherichia coli cloning vector pHSG399 <400> SEQUENCE: 6 gatatctacg tgccgatcaa cgtctc 26 <210> SEQ ID NO 7 <211> LENGTH: 25 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence:primer for amplifying Escherichia coli cloning vector pHSG399 <400> SEQUENCE: 7 aggccttttt ttaaggcagt tattg 25